Associated products

Product	Description	Pack Size	Cat No.
ISOLATE II Genomic DNA Kit	Rapid isolation of high-quality genomic DNA from a wide variety of samples	10 Preps 50 Preps 250 Preps	BIO-52065 BIO-52066 BIO-52067
ISOLATE II Plant DNA Kit	Rapid isolation of high-quality genomic DNA from a wide variety of plant species	10 Preps 50 Preps 250 Preps	BIO-52068 BIO-52069 BIO-52070
EPIK Fast Quantification Lo-ROX Kit	Highly reproducible and sensitive real-time PCR with bisulfite-modified DNA templates	200 Reactions 500 Reactions	BIO-66027 BIO-66028
EPIK Amplification Kit	Highly reproducible and sensitive PCR with bisulfite-modified DNA templates	200 Reactions 500 Reactions	BIO-66025 BIO-66026
DEPC-treated Water	Deionized, high-quality molecular grade water treated with DEPC. Ideal for use in all RNA work	10 x 10ml 1 Liter	BIO-38030 BIO-38031

Technical support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

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Trademark and licensing information

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Storage and Stability:
The EPIK™ Fast Quantification Hi-ROX Kit is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Quality control:
Bioline operates under ISO 9001 Management System. The EPIK Fast Quantification Hi-ROX Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Safety precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Notes:
For research use only.

EPIK™ Fast Quantification Hi-ROX Kit

Shipping: On dry/blue ice

Batch No.: See vial

Concentration: See vial

Catalog numbers

BIO-66029: 200 x 20µl reactions: 2 x 1ml

BIO-66030: 500 x 20µl reactions: 5 x 1ml

BIOLINE
A Meridian Life Science® Company

Store at –20°C

Description

The EPIK Fast Quantification Hi-ROX Kit has been developed to overcome the challenges associated with the quantification of bisulfite -modified DNA templates, to give highly reproducible and ultra-sensitive real-time PCR results. EPIK Fast Quantification Hi-ROX Kit contains EPIKORET buffer, built on the latest advances in buffer chemistry and enhancers, together with SensiFASTT, an antibody-mediated hot-start DNA polymerase. This ensures that the EPIK Fast Quantification Hi-ROX Kit delivers the high speed and enhanced specificity needed for high-throughput epigenetic analysis.

Kit components

Reagent	200 x 20µl Reactions	500 x 20µl Reactions
EPIK Fast Quantification Hi-ROX mix (2x)	2 x 1ml	5 x 1ml

General considerations

To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primers: The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your real-time PCR:

- When designing primers it is important to consider that bisulfite treated-DNA is no longer double stranded and the primer set has to be specifically designed for the forward or the reverse strands. For that purpose online programs such as *Methprimer* (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi) or *BiSearch* (http://bisearch.enzim.hu/?m=search) are valuable tools.

- The optimal amplicon length should be 80-200bp and should not exceed 400bp

- Primers should have a melting temperature (Tm) of approximately 60°C

- We recommend final primer concentrations of 0.4µM (*i.e.* 8pmol of each primer per 20µl reaction volume). If necessary, the primer concentrations in the range 0.1-1µM can be optimized by titration

Template: It is important that the DNA template is suitable for use in PCR in terms of purity and concentration. The template needs to be devoid of any contaminating PCR inhibitors (e.g. EDTA). Up to 500ng of template can be required in a single PCR. The suggested initial amount is 100ng of converted DNA per reaction; however it may be necessary to vary this amount.

The amount of template in the reaction depends mainly on the source of DNA as well as the conversion kit used. Higher amounts of template (up to 200ng per reaction) are required for DNA with high structural complexity such as that from eukaryotes. In addition, when the template has been treated with conversion kits with a greater tendency to fragment the DNA, a higher amount of template is required.

The converted DNA has similar optical properties to single-stranded RNA because it has both high uracil content and is mostly made up of non-complementary nucleic acid which can be considered as single-stranded. Quantification should be performed with methods adapted to single stranded nucleic acid analysis (e.g. Nanodrop with RNA settings). We suggest using a value of 40µg/ml^{-1} for an OD_{260} of 1.0 when determining the concentration of converted DNA.

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM which is optimal for the hot-start DNA polymerase in the majority of real-time PCR conditions. In some instances, increasing the final $MgCl_2$ concentration to 6mM will reduce C_t values for difficult amplicons.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-template control reaction, replacing the template with PCR grade water. We also recommend including a no-conversion control where the converted template is substituted by the non-converted DNA. This will check for the specificity of the primers used.

Instrument compatibility

The EPIK Fast Quantification Hi-ROX Kit can be used on instruments that do not require the use of ROX (5-carboxy-X-rhodamine, single isomer), such as the BioRad® Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384, iQ5™, Cepheid® SmartCycler™, Qiagen (Corbett) Rotor-Gene™ 3000, 6000 & Q, Analytik Jena qTower2, Eppendorf Mastercycler ep Realplex, ep Realplex 2S, Roche LightCycler® 480, LightCycler® Nano, Techne Quantica®, PrimeQ, Illumina Eco™, Takara Thermal Cycler Dice® TP800.

Optionally, the EPIK Fast Quantification Hi-ROX Kit can also be used in real-time PCR on some instruments (such as the ABI/ Invitrogen 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™ and StepOne™ Plus) that offer the user the choice of analyzing the real-time PCR data with the passive reference signal either on or off. If your real-time instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software *(see notice to purchaser Trademark and Licensing Information, point 6)*.

Procedure

Reaction set-up: The following protocol is for a standard 20µl reaction and can be scaled accordingly. Prepare a PCR mastermix as described below.

Reagent	Volume	Final
2x EPIK Fast Quantification Hi-ROX mix	10µl	1x
Primers (10µM each)	0.8µl	400nM (each)
Template	up to 9.2µl	<500ng
H_2O	As required	
	20µl Final volume	

Troubleshooting guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	For cDNA templates, make sure EPIK Fast Quantification Hi-ROX is activated for 2min at 95°C before cycling.
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template
	Incorrect concentration of primers	Use primer concentration between 100nM and 1µM
	Template degraded or fragmented	Re-prepare converted DNA or use another conversion kit supplier
	Primers degraded	Use newly synthesized primers
	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade H_2O
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C

Sensitivity testing and C_t values: When comparing EPIK Fast Quantification Hi-ROX with a mix from another supplier we recommend running the different mixes on separate plates. In addition, we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early C_t value is not an indication of good sensitivity, but rather an indication of speed.

The following real-time PCR conditions are suitable for the EPIK Fast Quantification Hi-ROX Kit with amplicon sizes up to 200bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures below 60°C or combined annealing/ extension times longer than 30 seconds.

Cycles	Temp.	Time	Notes
1	95°C	2min	Polymerase activation
40-45	95°C	5s	Denaturation
	65°C	10s	Annealing
	72°C	5-20s*	Extension (acquire at end of step)

*** We do not recommend extending beyond 20 seconds**

Optional analysis: After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Suboptimal primer design	Redesign primers using appropriate software or use validated primers
	Primer concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primer concentration too low	Titrate primers in the concentration range of 100nM - 1µM
	Primer annealing temperature too low	Due to the high ionic strength of EPIK Fast Quantification Hi-ROX Kit it is not recommended to use annealing/extension temperatures below 60°C. Annealing/extension temperature can be increased in steps of 2°C in the event of non-specific products
	Template concentration too low	Increase template concentration
	Template concentration too high	Reduce template concentration until non-specific products disappear
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Variability between replicates	Error in reaction set-up	Prepare large volume mastermix, vortex thoroughly and aliquot into reaction plate
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Activation time too short	Ensure the reaction is activated for between 1min and 3min at 95°C before cycling
	Extension time too short	Increasing the extension time may be necessary for amplification products over 200bp; double extension time to determine whether the cycle threshold (C_t) is affected
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C
	Template concentration too low	Increase concentration if possible
	High fragmentation of template	Increase the amount of template Re-prepare converted DNA or use another conversion kit supplier
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
	Primer concentration too low	Increase concentration of primer in 100nM increments
	$MgCl_2$ concentration insufficient	Increase final $MgCl_2$ concentration to 6mM
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primer concentration too low	Increase concentration of primer in 100nM increments
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers
PCR efficiency above 110%	Template is degraded or contains PCR inhibitors	Re-prepare template Use another conversion kit supplier Purify template and resuspend in water
	Non-specific amplification and/or primer-dimers	Use melt analysis and 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products